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Photosynthetic Machinery in Relation to Leaf Agro-Histological Traits of Ten Wheat Genotypes Facing Drought

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ABSTRACT

A semi-field experiment was conducted to assess the response of ten wheat (*Triticum aestivum* L.) genotypes to drought applied at booting stage by withholding 25% of field capacity for three weeks. Flag leaves were checked for their performance under water sufficient and deficient conditions. Split plot statistical analysis of data revealed that irrespective of genotype, drought generally caused marked decrease in leaf biomass, area, water content and succulence; with non-significant effect on leaf specific area and sclerophylly. Conversely, drought increased leaf thickness as well as the area of xylem and whole vascular bundle, while that of phloem was non-significantly affected. Irregular shape of mesophyll chloroplasts was identified in leaves of drought plants with unorganized membranous system, less starch grains and more plastoglobules compared with their well-watered synonyms. Moreover, drought significantly decreased photosynthesis and transpiration rate as well as stomatal and mesophyll conductance; with non-significant effect on photosynthetic water use efficiency, internal CO_2 concentration and stomatal limitation. Significant decrease in polysaccharides was also recorded to accompany the decrease in chlorophyll a and chlorophyll a/b ratio under drought; with marked increase in glucose, fructose, sucrose, trehalose, total soluble sugars, total carbohydrates as well as chlorophyll b, total chlorophyll, carotenoids and chlorophyll stability index. Furthermore, variations among genotypes irrespective of watering level besides their individual responses to drought are specifically discussed. Also, drought-induced changes in the estimated parameters are correlated. Generally, the Sids genotypes and Shandawel 1 seemed to have the best leaf agro-histological features and the most efficient photosynthetic machinery when droughted.

Keywords: Drought, Gas exchange, Leaf anatomy, Photosynthetic efficacy, Ultrastructure, Wheat

Abbreviations: A: Photosynthesis Rate; C_a: Ambient CO_2 Concentration; C_i: Intercellular CO_2 Concentration; CSI: Chlorophyll Stability Index; E: Transpiration Rate; g_m: Mesophyll Conductance; g_s: Stomatal Conductance; L_s: Stomatal Limitation; pWUE: Photosynthetic Water Use Efficiency

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the world's most widely cultivated crop that occupies about one-third of the cereal cultivation area to contribute more than 20% of the total caloric intake to humans [1]. By the year 2030, global wheat production may need to be upgraded by at least 50% to feed the continuously growing population [2]. Such goal is not to somewhat easy since the rate of annual growth in wheat production was documented to fall down from 3% to less than 1% in the past few years [3]. Among the major constraints for crop yield, drought was recorded to severely limit wheat production all over the world; but more notably in arid and semi-arid regions [4]. At the beginning of the twenty first century, it was reported that 70% of the global area cultivated with wheat had experienced water stress [5]. Therefore, identifying drought-tolerant wheat genotypes and understanding the mechanisms of their ability to withstand in dry habitats may be a strategic way to increase wheat productivity.

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Although water deficit could impede wheat performance at almost all growth stages, it is more critical during booting stage when flag leaf sheath swells just before heading; leading to severe drop in yield. According to source-sink relationship, wheat leaves and more importantly the flag one have a large share in the net yield since the leaves are the plant parts mainly responsible for photosynthesis and production of photo-assimilates that would be allocated into different plant parts till reaching the developed grains. Under water stress conditions, lower rate of net photosynthesis was intensively recorded owing to oxidative damage to chloroplasts and the associated pigment molecules as well as stomatal limitation with great disturbance in leaf gas exchange parameters leading to poor grain set and development [6].

Not only the plant growth stage at which stress is applied and the stress severity are the determinant of wheat response to drought, but it is also the genotype that greatly determines the degree of tolerance or susceptibility to stress. Thus, there is an urgent need to identify genotypes with reasonable vegetative traits contributing to improved yield under control and drought conditions [7]. Therefore, the present investigation was designed to evaluate the effect of drought on ten wheat genotypes at booting stage. For that, some agronomic, anatomical and ultra-structural features of their flag leaves in relation to their photosynthetic pigmentation system, gas exchange parameters and photo-assimilates were assessed. In addition, intensive statistical analysis of the data obtained was employed in order to elucidate the solo effect of each of the watering level and the genotype as well as their combined effect on the concerned plants. Correlations among the drought-induced changes in the estimated parameters were also computed along with statistical ranking of the addressed traits and genotypes according to their response to drought.

MATERIALS AND METHODS

Plant materials and experimental design

Pure strains of wheat (*Triticum aestivum* L.) genotypes Masr 1, Masr 2, Gimmaza 9, Gimmaza 11, Sids 12, Sids 13, Sakha 93, Sakha 94, Shandawel 11 and Giza 186 were obtained from the Egyptian Ministry of Agriculture to be cultivated within plastic pots packed with 10 kg soil (clay/sand, 2/1, v/v) till thinning into 5 uniform seedlings after 30 days. The pots were kept in a greenhouse under natural conditions suitable for plant growth and development. All plants were irrigated to field capacity for 45 days then plants from each genotype were categorized into two sets; the first was still normally irrigated serving as control, whereas irrigation was withheld from the second set for 21 days in such a way that 25% of irrigation water was held. When the plants were 65 day old, sampling of the flag or uppermost leaf was carried out.

Estimation of leaf agronomy

Growth vigor of the uppermost leaf was estimated. Some leaf agronomic features, such as fresh and dry weight, were directly scored; while others could be calculated according to the following relations:

Water content = (fresh mass - dry mass) / fresh mass

Succulence degree = (fresh mass - dry mass) / area [8]

Sclerophylly degree = dry mass / area [8]

Succulence quotient = succulence degree / sclerophylly degree [9]

Estimation of leaf anatomy

Firstly leaf area and specific area were determined as following:

Leaf area = length × breadth × 0.75 [10]

Specific area = area / dry mass [11]

Leaves were then fixed in formalin: acetic acid: ethanol (1: 1: 18, v/v) for 48 h. Dehydration, clearing, staining and mounting procedures were followed as recommended by Maiti et al. [12]. Sections were examined under light microscope, photographed and analyzed using "Image J" version 1.38 software.

Estimation of leaf ultrastructure

Transmission electron microscopy was carried out following Reynolds [13]. Square sections of leaves were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde buffered in 0.1 M sodium phosphate buffer (pH 7.4). The plant tissues were then processed, cut into ultra-thin sections and rinsed into copper grids to be examined and photographed at 4000x using JEOL JEM-2100 transmission electron microscope at Electron Microscopy Unit, Mansoura University, Egypt.

Estimation of photosynthetic pigments

A known fresh weight of leaves was macerated in 80% chilled acetone in presence of solid traces of $MgCO_3$, centrifuged and the supernatant was raised to a total volume. The absorbance (A) was measured at 3 wavelengths to calculate the amount of photosynthetic pigments in µg ml^{-1} as following:

Chlorophyll a = 10.3 A663 - 0.918 A644

Chlorophyll b = 19.7 A644 - 3.87 A663

Carotenoids = 5.02 A480

Pigment fractions were finally expressed as µg mg^{-1} f wt [14,15]. In addition, total chlorophyll (chlorophyll a+b), chlorophyll a/b and carotenoids/total chlorophyll were calculated. Also, chlorophyll stability index (CSI) was recorded following Sairam et al. [16] as following:

CSI = 100 × total chlorophyll Drought / total chlorophyll Control

Estimation of leaf gas exchange

Portable gas exchange system (LCi, ADC Bio Scientific, UK) was used to measure some photosynthetic parameters in situ using open flow mode. Leaves were oriented normally to the incoming radiation with average photosynthetically active radiation of 700 µmol m^{-2} s^{-1}, temperature of 28°C and ambient CO_2 concentration (Ca) of 360 µmol mol^{-1} within the chamber. Various gas exchange parameters like photosynthesis rate (A), transpiration rate (E), intercellular CO_2 concentration (C_i) and stomatal conductance (g_s) could be directly measured. In addition, other parameters were calculated as following:

Photosynthetic water use efficiency (pWUE) = A / E

Mesophyll conductance (gm) = A / Ci

Stomatal limitation (Ls) = 1- (Ci / Ca) [17]

Estimation of carbohydrates

A known dry weight of leaves was extracted with 80% ethanol; and the alcoholic extracts were used to colorimetrically determine the amount of glucose using O-toluidine reagent [18], fructose using resorcinol reagent [19], sucrose and total soluble sugars using anthrone reagent [20,21]. Meanwhile, acidic extracts; trichloroacetic acid extracts for trehalose as recommended by Fu et al. [22] and perchloric ones for polysaccharides as recommended by Sadasivam and Manickam [23], were used along with anthrone reagent for both.

STATISTICAL ANALYSIS

Five replicates were taken to assess the agronomic traits, while only three were used for histological and biochemical assays. "CoHort/CoStat" version 6.311 software was employed to analyze data with two sets of analyses. The first set was mainly descriptive to calculate the means and standard deviations. The second one comprised an analysis of variance (ANOVA) at $p \leq 0.05$ with one way completely randomized (1WCR) and split plot (SP) designs; and the degree of significance was referred to as ***, **, * or ns for respective high, medium, low or non-significant variation. Superscript letters were given so that different superscripts indicate significant variation. To assess the impact of drought on each of the estimated parameters, impact index was calculated based on the SP outputs as:

100 × (drought value - control value) / control value

Pearson correlation coefficient (r) was recorded among the drought-induced changes in the estimated parameters then heat map was illustrated to indicate r values. Stress impact coefficient (SIC) was also calculated to derive stress impact index (SII) for the addressed traits (SIItrait) and genotypes (SIIgenotype) to rank them according to their response to drought [24].

RESULTS AND DISCUSSION

Leaf agronomic traits are among the plant criteria mostly affected by drought. According to SP analysis of data in **Table 1**, wheat genotype Sakha 93 seemed to show the maximum leaf biomass and water content regardless of watering level. SP analysis revealed also that drought significantly reduced leaf biomass and water content regardless of genotype. According to 1WCR analysis in **Table 1**, drought significantly decreased leaf fresh mass of all genotypes except for Shandawel 1 and Giza 186 where the recorded decrease was non-significant. Regarding the drought-induced decrease in leaf dry mass, only Masr 1, Gimmaza 9 and Sids (the two studied genotypes) responded by non-significant decrease in their leaf dry mass. For leaf water content, the drought-induced decrease was significant only in five genotypes (Masr, Gimmaza and Sakha 94). The results recorded herein for leaf agronomic traits of wheat plants under drought agree with those recorded by Aldesuquy et al. [25]. The recorded decrease in leaf biomass and water content may be attributed to drought-induced: (i) drop in cellular turgor pressure that inhibits cell division, enlargement and differentiation [26], (ii) little assimilates supply caused by imposed constraints on plant processes especially photosynthesis [27], (iii) interference with nutrient availability which accompanies little water supply [28], and/or, (iv) delayed leaf emergence and early leaf senescence [29]. From another point of view, decreased leaf biomass under drought can be considered as an adaptive response of the studied wheat plants to cope with water deficit. In this regard, it was supposed that the first strategy maintained by some plants to control water loss is to restrict leaf growth [30].

Regarding leaf succulence, two measures of succulence can be indicative; and these include succulence degree and succulence quotient. Succulence degree (water amount per unit leaf area) usually indicates an adaptation to drought; where succulent organs have more capacity to store water. Succulence quotient (water amount per unit organic matter) however allows better understanding of how much energy a leaf uses to reserve water [8]. According to SP analysis of data in **Table 1**, wheat genotype Sakha 93 seemed to show the maximum leaf succulence degree and quotient. Irrespective of genotype, SP analysis revealed that drought significantly decreased leaf succulence degree and quotient of wheat plants. From 1WCR analysis, significant decrease in each of succulence degree and quotient was recorded in the genotypes Masr, Gimmaza and Sakha; but non-significant decrease was recorded in the remaining four genotypes **(Table 1)**. Nevertheless, the least decrease in succulence degree and quotient was recorded for wheat genotype Sids 13 and Sids 12, while the highest decrease was recorded for Gimmaza 11 and Gimmaza 9. So, the two

Sids genotypes appear to be less susceptible to drought than the two Gimmaza ones when considering their leaf succulence. Correlation among the traits of the ten genotypes revealed that the recorded drought-induced change in leaf succulence degree and that in succulence quotient were very strongly correlated with each other (r=1). Moreover, the drought- induced change in leaf succulence (indicated by succulence degree or quotient) was strongly correlated with the change in leaf water content (r=0.93 for each) **(Figure 1)**. Matching these results, Torrecillas et al. [31] recorded marked decrease in leaf succulence of tomato plants with corresponding decrease in leaf water content as a result of drought.

Table 1. Effect of drought on leaf agronomic features of ten wheat genotypes.

		Fresh Weight (mg)	Dry Weight (mg)	Water Content (mg H_2O g^{-1} f wt)	Succulence Degree (mg cm^{-2})	Sclerophylly Degree (mg cm^{-2})	Succulence Quotient (mg H_2O mg^{-1} d wt)
Factor AB: Genotype × Watering Level							
Masr 1	Control	$430^{b} \pm 113$	$107^{bcdef} \pm 19$	$745^{ab} \pm 39$	$15.1^{abc} \pm 1.4$	$5.2^{c} \pm 0.7$	$3.01^{ab} \pm 0.75$
	Drought	$251^{hij} \pm 32$	$77^{hi} \pm 9$	$693^{cdef} \pm 6$	$11.9^{f} \pm 0.8$	$5.3^{bc} \pm 0.3$	$2.25^{efgh} \pm 0.06$
Masr 2	Control	$361^{bcdef} \pm 65$	$96^{efgh} \pm 16$	$732^{abcd} \pm 7$	$15.0^{abc} \pm 1.7$	$5.5^{bc} \pm 0.5$	$2.73^{abcd} \pm 0.10$
	Drought	$239^{ij} \pm 24$	$76^{hi} \pm 7$	$681^{ef} \pm 5$	$11.8^{f} \pm 0.6$	$5.5^{bc} \pm 0.2$	$2.14^{gh} \pm 0.05$
Gimmaza 9	Control	$422^{bc} \pm 52$	$114^{abcde} \pm 10$	$730^{abcde} \pm 12$	$15.8^{ab} \pm 1.0$	$5.8^{abc} \pm 0.2$	$2.70^{abcde} \pm 0.17$
	Drought	$261^{ghij} \pm 109$	$87^{fghi} \pm 23$	$629^{gh} \pm 131$	$12.0^{f} \pm 4.1$	$6.5^{a} \pm 0.6$	$1.89^{hi} \pm 0.70$
Gimmaza 11	Control	$408^{bcde} \pm 82$	$126^{ab} \pm 33$	$695^{bcdef} \pm 29$	$13.4^{cdef} \pm 2.6$	$5.8^{abc} \pm 0.9$	$2.30^{defgh} \pm 0.34$
	Drought	$311^{fghi} \pm 69$	$118^{abcd} \pm 16$	$613^{h} \pm 70$	$9.4^{g} \pm 1.8$	$5.9^{ab} \pm 0.9$	$1.64^{i} \pm 0.41$
Sids 12	Control	$372^{bcdef} \pm 71$	$111^{abcde} \pm 14$	$698^{bcdef} \pm 18$	$12.4^{ef} \pm 1.1$	$5.3^{bc} \pm 0.4$	$2.32^{defgh} \pm 0.21$
	Drought	$262^{ghij} \pm 40$	$79^{ghi} \pm 12$	$696^{bcdef} \pm 17$	$12.3^{ef} \pm 1.0$	$5.3^{bc} \pm 0.5$	$2.30^{defgh} \pm 0.20$
Sids 13	Control	$371^{bcdef} \pm 42$	$101^{cdef} \pm 12$	$725^{abcdef} \pm 33$	$14.1^{abcde} \pm 1.3$	$5.4^{bc} \pm 0.9$	$2.68^{abcde} \pm 0.44$
	Drought	$259^{ghij} \pm 20$	$72^{i} \pm 7$	$721^{abcdef} \pm 23$	$14.0^{bcde} \pm 1.1$	$5.4^{bc} \pm 0.4$	$2.60^{bcdef} \pm 0.28$
Sakha 93	Control	$520^{a} \pm 68$	$128^{a} \pm 4$	$750^{a} \pm 37$	$16.0^{a} \pm 1.4$	$5.3^{bc} \pm 0.7$	$3.07^{a} \pm 0.63$

	Drought	$420^{bcd} \pm 92$	$121^{abc} \pm 26$	$711^{abcdef} \pm 23$	$13.2^{cdef} \pm 1.0$	$5.4^{bc} \pm 0.2$	$2.48^{cdefg} \pm 0.27$
Sakha 94	Control	$398^{bcde} \pm 34$	$103^{cdef} \pm 6$	$740^{abc} \pm 28$	$14.7^{abcd} \pm 0.9$	$5.2^{c} \pm 0.6$	$2.87^{abc} \pm 0.40$
	Drought	$221^{j} \pm 16$	$70^{i} \pm 8$	$685^{def} \pm 34$	$12.0^{f} \pm 1.9$	$5.5^{bc} \pm 0.6$	$2.21^{fgh} \pm 0.38$
Shandawel 1	Control	$338^{defg} \pm 29$	$100^{defg} \pm 11$	$705^{abcdef} \pm 9$	$13.4^{cdef} \pm 0.5$	$5.6^{bc} \pm 0.3$	$2.39^{defg} \pm 0.10$
	Drought	$328^{efgh} \pm 86$	$100^{defg} \pm 26$	$697^{bcdef} \pm 20$	$13.0^{def} \pm 0.9$	$5.7^{bc} \pm 0.6$	$2.31^{defgh} \pm 0.21$
Giza 186	Control	$369^{bcdef} \pm 59$	$116^{ab} \pm 18$	$684^{def} \pm 19$	$12.2^{ef} \pm 0.6$	$5.6^{bc} \pm 0.4$	$2.18^{fgh} \pm 0.18$
	Drought	$345^{cdef} \pm 67$	$111^{abcde} \pm 18$	$676^{fg} \pm 17$	$12.0^{f} \pm 1.0$	$5.7^{bc} \pm 0.3$	$2.09^{ghi} \pm 0.15$
Factor A: Genotype							
Masr 1		$341^{bc} \pm 122$	$92^{bc} \pm 21$	$719^{a} \pm 38$	$13.5^{ab} \pm 2.0$	$5.2^{d} \pm 0.5$	$2.63^{abc} \pm 0.64$
Masr 2		$300^{c} \pm 79$	$86^{c} \pm 16$	$706^{ab} \pm 27$	$13.4^{ab} \pm 2.1$	$5.5^{bcd} \pm 0.3$	$2.43^{bcd} \pm 0.32$
Gimmaza 9		$341^{bc} \pm 117$	$100^{b} \pm 22$	$679^{bc} \pm 102$	$13.9^{a} \pm 3.4$	$6.2^{a} \pm 0.6$	$2.30^{cde} \pm 0.64$
Gimmaza 11		$360^{b} \pm 88$	$122^{a} \pm 25$	$654^{c} \pm 66$	$11.4^{c} \pm 3.0$	$5.9^{ab} \pm 0.9$	$1.97^{e} \pm 0.49$
Sids 12		$317^{bc} \pm 79$	$95^{bc} \pm 21$	$697^{ab} \pm 17$	$12.3^{bc} \pm 1.0$	$5.3^{cd} \pm 0.4$	$2.31^{bcd} \pm 0.20$
Sids 13		$315^{bc} \pm 67$	$86^{c} \pm 18$	$723^{a} \pm 27$	$14.0^{a} \pm 1.1$	$5.4^{cd} \pm 0.7$	$2.64^{ab} \pm 0.35$
Sakha 93		$470^{a} \pm 93$	$125^{a} \pm 18$	$731^{a} \pm 36$	$14.6^{a} \pm 1.9$	$5.3^{cd} \pm 0.5$	$2.77^{a} \pm 0.56$
Sakha 94		$310^{bc} \pm 96$	$86^{c} \pm 19$	$713^{ab} \pm 41$	$13.3^{ab} \pm 2.0$	$5.3^{cd} \pm 0.6$	$2.54^{abc} \pm 0.51$
Shandawel 1		$333^{bc} \pm 61$	$100^{b} \pm 19$	$701^{ab} \pm 15$	$13.2^{ab} \pm 0.7$	$5.6^{bc} \pm 0.4$	$2.35^{bcd} \pm 0.16$
Giza 186		$357^{b} \pm 61$	$114^{a} \pm 17$	$680^{bc} \pm 17$	$12.1^{bc} \pm 0.8$	$5.7^{bc} \pm 0.3$	$2.13^{de} \pm 0.16$
Degree of Significance		***	***	**	**	**	***

Factor B: Watering Level						
Control	$399^{a} \pm 77$	$110^{a} \pm 18$	$720^{a} \pm 32$	$14.2^{a} \pm 1.8$	$5.5^{a} \pm 0.6$	$2.63^{a} \pm 0.46$
Drought	$290^{b} \pm 82$	$91^{b} \pm 24$	$680^{b} \pm 56$	$12.1^{b} \pm 2.0$	$5.6^{a} \pm 0.6$	$2.19^{b} \pm 0.40$
Degree of Significance	***	***	***	***	ns	***
Impact Index (%)	-27	-17	-6	-15	2	-17

Data listed represent mean values ± standard deviation. Different superscript letters refer to significant variation at $p \leq 0.05$. Low, medium and high degree of significance is indicated by *, ** and *** while non-significant difference is abbreviated as ns

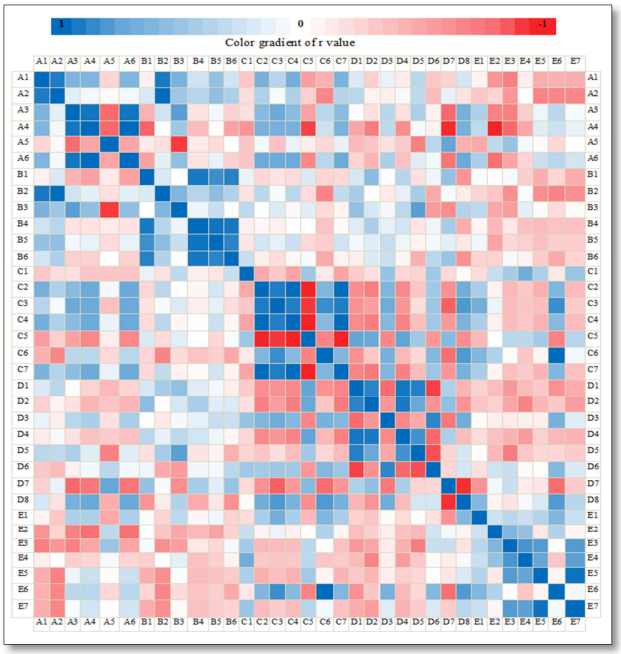

Figure 1. Heat Map of Pearson correlation coefficient among the percent of change in the estimated leaf traits of ten wheat genotypes in response to drought. (A1: leaf fresh weight, A2: dry weight, A3: water content, A4: succulence degree, A5: sclerophylly degree, A6: succulent quotient, B1: leaf thickness, B2: area, B3: specific area, B4: vascular bundle area, B5: xylem area, B6: phloem area, C14: leaf chlorophyll a content, C2: chlorophyll b content, C3: carotenoids content, C4: total chlorophyll content, C5: chlorophyll a/b ratio, C6: carotenoids/total chlorophyll ratio, C7: chlorophyll stability index, D1: photosynthesis rate, D2: transpiration rate, D3: photosynthetic water use efficiency, D4: stomatal conductance, D5: mesophyll conductance, D6: intercellular CO2 concentration/stomatal conductance, D7: intercellular CO2 concentration, D8: stomatal limitation, E1: leaf glucose content, E2: fructose content, E3: sucrose content, E4: total soluble sugars content, E5: trehalose content, E6: polysaccharides content, E7: total carbohydrates content).

On contrary, SP analysis in **Table 1** revealed that drought non-significantly increased leaf sclerophylly degree (dry mass per unit leaf area); with the two Gimmaza genotypes exhibiting the maximum leaf sclerophylly. Also, 1WCR data analysis revealed that drought caused non-significant increase in leaf sclerophylly in all genotypes (**Table 1**). In this connection, Edwards et al. [32] discussed the significance of sclerophylly on the basis of three hypotheses that center on sclerophylly as: (i) an adaptation to water stress, (ii) an adaptation to, or consequence of, low nutrient supply and (iii) improvement of leaf longevity by protecting leaf and increasing its carbon gain. Correlation among the traits addressed herein revealed that the recorded drought-induced change in leaf sclerophylly of the ten genotypes was strongly and negatively correlated with the change in leaf specific area (r=-0.88) (**Figure 1**); indicating that the recorded non-significant increase in leaf sclerophylly in response to drought can be attributed to the recorded non-significant decrease in leaf specific area. Matching these results, Chartzoulakis et al. [33] recorded that olive plants could overcome water deficit with by increasing leaf sclerophylly.

Furthermore, leaf anatomical features are clear indicators for plant performance whether under control or stress conditions. Results of SP analysis in **Table 2** cleared that irrespective of watering level, wheat genotypes Masr 1 and Sakha 93 had the maximum leaf thickness, area and specific area. Irrespective of genotype, drought caused highly significant increase in leaf thickness, highly significant decrease in leaf area and non-significant change in leaf specific area all at $p \leq 0.05$. Applying 1WCR data analysis revealed that drought caused significant decrease in leaf thickness of all the studied genotypes except for Masr 2, Gimmaza 11, Sakha 94 and Giza 186 where significant increase in leaf thickness was recorded in response to drought. Also, drought decreased leaf area in all genotypes; but such reduction was significant only in Masr 1, Gimmaza 9, Sids and Sakha 94. As mentioned before, the reduction reported in leaf specific area in response to drought was statistically non-significant in all the studied genotypes (**Table 2**). Drought-induced changes in leaf thickness, area

and specific area were previously reported in other investigations [34], where anatomical alterations may occur in plants under water stress either as a negative impact of stress or as an adaptive feature. Generally, decreased leaf area and specific area under stress conditions can be attributed to the same reasons causing reduction in leaf biomass. In this regard, the recorded drought-induced decrease in leaf area of the ten genotypes was found to be strongly correlated with the drought-induced decrease in leaf biomass (r=0.90 with leaf fresh mass and r=0.99 with dry mass) **(Figure 1)**. Alternatively, such decrease in leaf growth may be a powerful means to reduce the transpirative surface and thus saving as much water as possible; and may also conserve carbohydrates along with other energy resources.

Table 2. Effect of drought on leaf anatomical features of ten wheat genotypes.

		Leaf Thickness (µm)	Leaf Area (cm^2)	Leaf Specific Area ($cm^2\ g^{-1}$ d wt)	Vascular Bundle Area (mm^2)	Xylem Area (mm^2)	Phloem Area (mm^2)
Factor AB: Genotype × Watering Level							
Masr 1	Control	$431^{bcd} \pm 98$	$21.1^{abc} \pm 4.7$	$197^{a} \pm 30$	$0.011^{ef} \pm 0.000$	$0.008^{ab} \pm 5.8\times10^{-4}$	$0.003^{a} \pm 0.000$
	Drought	$513^{a} \pm 20$	$14.6^{def} \pm 1.0$	$190^{abc} \pm 9$	$0.008^{ij} \pm 5.8\times10^{-4}$	$0.004^{fg} \pm 5.8\times10^{-4}$	$0.002^{bc} \pm 0.000$
Masr 2	Control	$247^{hij} \pm 34$	$17.5^{cde} \pm 1.8$	$183^{abc} \pm 16$	$0.007^{k} \pm 0.001$	$0.004^{g} \pm 0.000$	$0.002^{bc} \pm 0.000$
	Drought	$390^{def} \pm 16$	$13.8^{ef} \pm 0.9$	$182^{abc} \pm 6$	$0.009^{hi} \pm 0.001$	$0.005^{def} \pm 0.000$	$0.002^{ab} \pm 5.8\times10^{-4}$
Gimmaza 9	Control	$448^{b} \pm 6$	$19.5^{bc} \pm 1.5$	$172^{cd} \pm 5$	$0.014^{a} \pm 0.001$	$0.008^{a} \pm 0.001$	$0.002^{bc} \pm 0.000$
	Drought	$399^{cde} \pm 16$	$13.6^{f} \pm 4.1$	$155^{d} \pm 13$	$0.013^{bc} \pm 5.8\times10^{-4}$	$0.007^{bc} \pm 5.8\times10^{-4}$	$0.002^{bc} \pm 0.000$
Gimmaza 11	Control	$216^{ijk} \pm 14$	$21.8^{ab} \pm 5.9$	$175^{abcd} \pm 26$	$0.007^{k} \pm 0.000$	$0.004^{g} \pm 0.000$	$0.001^{d} \pm 0.000$
	Drought	$442^{bc} \pm 18$	$20.2^{bc} \pm 3.4$	$173^{bcd} \pm 24$	$0.012^{bcd} \pm 5.8\times10^{-4}$	$0.008^{ab} \pm 5.8\times10^{-4}$	$0.002^{ab} \pm 5.8\times10^{-4}$
Sids 12	Control	$428^{bcd} \pm 9$	$21.1^{abc} \pm 3.8$	$189^{abc} \pm 13$	$0.010^{gh} \pm 5.8\times10^{-4}$	$0.005^{efg} \pm 5.8\times10^{-4}$	$0.001^{cd} \pm 5.8\times10^{-4}$
	Drought	$259^{hi} \pm 9$	$15.0^{def} \pm 3.2$	$188^{abc} \pm 16$	$0.008^{ij} \pm 5.8\times10^{-4}$	$0.004^{fg} \pm 5.8\times10^{-4}$	$0.001^{cd} \pm 5.8\times10^{-4}$
Sids 13	Control	$373^{ef} \pm 10$	$19.1^{bc} \pm 2.1$	$191^{abc} \pm 36$	$0.010^{fg} \pm 5.8\times10^{-4}$	$0.004^{fg} \pm 5.8\times10^{-4}$	$0.002^{bcd} \pm 5.8\times10^{-4}$
	Drought	$248^{hij} \pm 14$	$13.4^{f} \pm 0.7$	$187^{abc} \pm 15$	$0.009^{hi} \pm 0.000$	$0.005^{efg} \pm 5.8\times10^{-4}$	$0.001^{cd} \pm 5.8\times10^{-4}$
Sakha 93	Control	$346^{fg} \pm 18$	$24.3^{a} \pm 2.8$	$190^{abc} \pm 26$	$0.013^{ab} \pm 0.002$	$0.007^{c} \pm 0.001$	$0.002^{ab} \pm 5.8\times10^{-4}$
	Drought	$267^{h} \pm 2$	$22.5^{ab} \pm 4.3$	$187^{abc} \pm 8$	$0.011^{def} \pm 5.8\times10^{-4}$	$0.006^{d} \pm 5.8\times10^{-4}$	$0.002^{bc} \pm 0.000$
Sakha 94	Control	$205^{jk} \pm 17$	$20.0^{bc} \pm 1.7$	$195^{ab} \pm 22$	$0.006^{l} \pm 5.8\times10^{-4}$	$0.003^{h} \pm 0.000$	$0.001^{cd} \pm 5.8\times10^{-4}$
	Drought	$413^{bcde} \pm 8$	$12.8^{f} \pm 1.1$	$185^{abc} \pm 22$	$0.010^{fg} \pm 5.8\times10^{-4}$	$0.005^{de} \pm 5.8\times10^{-4}$	$0.002^{bc} \pm 0.000$
Sandawel 1	Control	$313^{g} \pm 4$	$17.8^{cd} \pm 1.6$	$179^{abc} \pm 9$	$0.009^{hi} \pm 0.000$	$0.004^{fg} \pm 5.8\times10^{-4}$	$0.002^{bc} \pm 0.000$
	Drought	$260^{hi} \pm 20$	$17.4^{cde} \pm 3.5$	$178^{abcd} \pm 18$	$0.008^{jk} \pm 5.8\times10^{-4}$	$0.005^{efg} \pm 5.8\times10^{-4}$	$0.002^{bcd} \pm 5.8\times10^{-4}$
Giza 186	Control	$198^{k} \pm 18$	$20.6^{abc} \pm 2.6$	$178^{abcd} \pm 12$	$0.006^{l} \pm 5.8\times10^{-4}$	$0.003^{h} \pm 0.000$	$0.001^{d} \pm 0.000$
	Drought	$378^{ef} \pm 15$	$19.5^{bc} \pm 3.3$	$175^{abcd} \pm 8$	$0.012^{cde} \pm 5.8\times10^{-4}$	$0.007^{bc} \pm 5.8\times10^{-4}$	$0.002^{ab} \pm 5.8\times10^{-4}$

Factor A: Genotype						
Masr 1	$472^{a} \pm 78$	$17.8^{de} \pm 4.7$	$194^{a} \pm 21$	$0.010^{b} \pm 0.002$	$0.006^{b} \pm 0.002$	$0.003^{a} \pm 5.5\times 10^{-4}$
Masr 2	$318^{abc} \pm 82$	$15.7^{e} \pm 2.4$	$183^{abc} \pm 11$	$0.008^{c} \pm 0.001$	$0.005^{de} \pm 5.5\times 10^{-4}$	$0.002^{ab} \pm 4.1\times 10^{-4}$
Gimmaza 9	$424^{d} \pm 29$	$16.5^{de} \pm 4.3$	$163^{d} \pm 13$	$0.013^{a} \pm 0.001$	$0.008^{a} \pm 9.8\times 10^{-4}$	$0.002^{abc} \pm 0.000$
Gimmaza 11	$329^{cd} \pm 125$	$21.0^{b} \pm 4.6$	$174^{cd} \pm 24$	$0.010^{b} \pm 0.003$	$0.006^{bc} \pm 0.002$	$0.002^{bcd} \pm 8.2\times 10^{-4}$
Sids 12	$343^{ab} \pm 93$	$18.1^{cd} \pm 4.6$	$188^{ab} \pm 14$	$0.009^{bc} \pm 8.9\times 10^{-4}$	$0.005^{de} \pm 5.5\times 10^{-4}$	$0.001^{d} \pm 5.2\times 10^{-4}$
Sids 13	$310^{ab} \pm 69$	$16.2^{de} \pm 3.3$	$189^{ab} \pm 26$	$0.010^{b} \pm 8.2\times 10^{-4}$	$0.005^{de} \pm 5.5\times 10^{-4}$	$0.002^{cd} \pm 5.5\times 10^{-4}$
Sakha 93	$306^{ab} \pm 45$	$23.4^{a} \pm 3.6$	$189^{ab} \pm 18$	$0.012^{a} \times 0.002$	$0.006^{b} \pm 9.8\times 10^{-4}$	$0.002^{ab} \pm 4.1\times 10^{-4}$
Sakha 94	$309^{ab} \pm 114$	$16.4^{de} \pm 4.1$	$190^{ab} \pm 21$	$0.008^{c} \pm 0.003$	$0.004^{e} \pm 0.001$	$0.002^{bcd} \pm 5.2\times 10^{-4}$
Shandawel 1	$287^{bc} \pm 32$	$17.6^{de} \pm 2.6$	$178^{bc} \pm 13$	$0.008^{c} \pm 8.2\times 10^{-4}$	$0.005^{de} \pm 5.5\times 10^{-4}$	$0.002^{bcd} \pm 4.1\times 10^{-4}$
Giza 186	$288^{bcd} \pm 100$	$20.1^{bc} \pm 2.9$	$177^{bcd} \pm 10$	$0.009^{bc} \pm 0.003$	$0.005^{cd} \pm 0.002$	$0.002^{bcd} \pm 8.2\times 10^{-4}$
egree of Significance	***	***	**	***	***	**
Factor B: Watering Level						
Control	$320^{b} \pm 100$	$20.3^{a} \pm 3.5$	$185^{a} \pm 21$	$0.009^{b} \pm 0.003$	0.005^{b} 0.002	$0.002^{a} \pm 6.8\times 10^{-4}$
Drought	$357^{a} \pm 90$	$16.3^{b} \pm 4.2$	$180^{a} \pm 17$	$0.010^{a} \pm 0.002$	$0.006^{a} \pm 0.001$	$0.002^{a} \pm 5.2\times 10^{-4}$
egree of Significance	***	***	ns	***	**	ns
Impact Index (%)	12	-20	-3	11	20	-1

Data listed represent mean values ± standard deviation. Different superscript letters refer to significant variation at $p \leq 0.05$. Low, medium and high degree of significance is indicated by *, ** and *** while non-significant difference is abbreviated as ns

Results of SP analysis in **Table 2** revealed also those wheat genotypes Gimmaza 9 and Sakha 93 appeared to generally have the maximum phloem, xylem and whole vascular bundle area. Irrespective of genotype, drought caused highly significant increase in vascular bundle area, moderately significant increase in xylem area but non-significant change in phloem area. Applying 1WCR analysis revealed that drought caused significant decrease in vascular bundle area with the same pattern of change in leaf thickness. Drought also caused significant decrease in xylem area in case of Masr 1, Gimmaza 9 and Sakha 93, while it caused significant increase in xylem area in Masr 2, Gimmaza 11, Sakha 94 and Giza 186. Otherwise, xylem area was non-significantly affected by drought. Significant increase in phloem area was recorded only in Gimmaza 11 and Giza 186; with non-significant change in the other genotypes in response to drought. Drought-induced changes in phloem, xylem and whole vascular bundle area of wheat leaves match those reported by Aldesuquy and Mickky [35]. The decrease recorded in phloem area as a result of drought, although being non-significant, may be a negative consequence of water deficit; where it was argued that the growth of phloem elements under water stress is likely to be reduced during cell development as a result of the reduced turgor-driven cell expansion [36]. Meanwhile, the increase recorded in vascular bundle and xylem area as well as in leaf thickness may be a strategy exerted by the studied wheat plants to cope with stress; where such increase can prevent excessive water loss and minimize injury resulting from dehydration, in addition to stimulating more effective water absorption and transport [34]. In this context, the recorded drought-induced change in leaf thickness of the ten genotypes was found to be strongly correlated with the change in vascular bundle area (r=0.91), xylem area (r=0.76) and phloem area (r=0.85). Also, the drought-induced change in bundle area of the ten genotypes was strongly correlated with the drought-induced change in each of xylem and phloem area (r=0.94 and 0.91, respectively) **(Figure 1)**.

In addition to leaf agronomic and anatomical features, drought is known to induce ultra-structural changes in plant leaves; with chloroplasts being one of the cellular organelles most affected by stress. In the present study, transmission

electron microscopy of the mesophyll cells in wheat flag leaves represented in **Figure 2** cleared that in genotype Masr 1, leaf chloroplasts of the control plants appeared lining cell wall with well-organized membranous system of grana, large starch grain or more appeared distinctly with almost no plastoglobules (Micrograph 1.a). When the plants of the same genotype were droughted, mesophyll chloroplasts were found relatively away from cell wall with irregular spherical shape, less-organized membrane system, almost no starch grains but plastoglobules began to be intensively formed in addition to some fat-storing bodies (oleosomes) within cells of the stressed plants (Micrograph 1.b). The same trend was approximately recorded in some other genotypes including Masr 2 (Micrographs 2), Gimmaza 9 (Micrographs 3), Sids 12 (Micrographs 5) and Sakha 93 (Micrographs 7) under control and stress conditions. In Gimmaza 11 (Micrographs 4) and Giza 186 (Micrographs 10), chloroplasts of the stressed plants were characterized by the presence of starch grains. In Sids 13 (Micrographs 6) and Shandawel 1 (Micrographs 9), chloroplasts of the stressed plants were characterized not only by the presence of starch grains but also by the formation of projections from some chloroplasts in the form of tails. In Sakha 94 (Micrographs 8), chloroplasts of the stressed plants do not contain starch grains but they were characterized by less number of plastoglobules.

General pattern of ultrastructural alterations in response to drought was similarly recorded elsewhere [37,38]. Disruption of leaf ultrastructure under stress is frequently attributed to over-production of reactive oxygen species (ROS); where chloroplasts are the major site for generating ROS as by-products of photosynthesis. Under normal conditions, ROS are produced in limited amounts as signaling molecules. However, ROS accumulated under stress are highly active causing oxidative stress; with cell membranes, organelles and biomolecules being their main targets [39]. As a consequence, serious membrane injury along with enzyme inactivation and organelles malformations are usually clear signs of stress. Also, the obvious appearance of plastoglobules (lipoprotein particles regulating plastid lipid metabolism within chloroplasts) is another indicator of stress [40]. Fewer starch grains within chloroplasts of droughted plants can be ascribed to: (i) less water supply that may suppress photosynthesis, (ii) adverse effect of ROS on photosystems and enzymes involved in photosynthesis, and/or, (iii) damage of starch grains by ROS. Fat-storing oleosomes may also appear, enlarge or increase in number to store lipids more assembled under stress [41].

Photosynthesis is also one of the main physiological processes adversely affected by drought; and the pigmentation system can provide direct indication for photosynthetic efficiency. Hence, it seemed so critical to assess the amount of photosynthetic pigments in wheat plants studied herein under the effect of drought. Data of SP analysis depending on genotype only in **Table 3** showed that

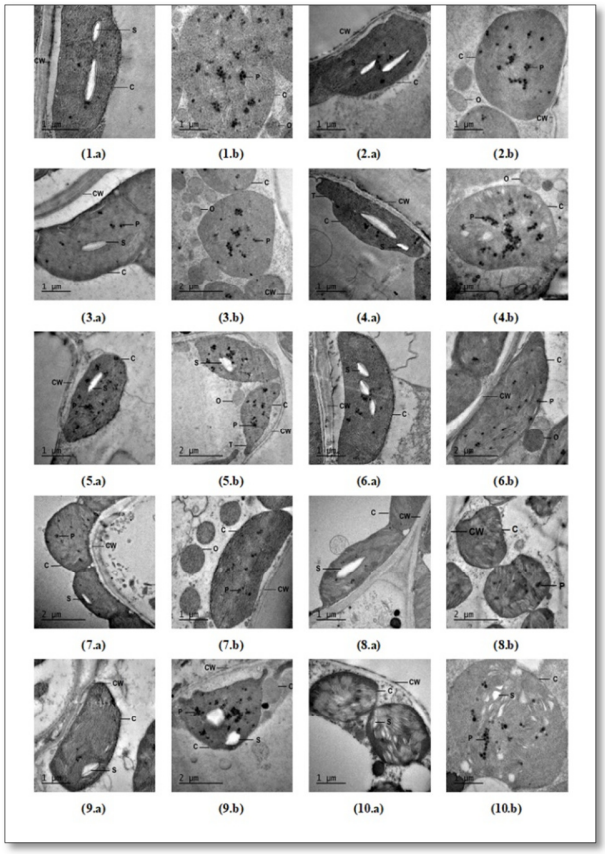

Figure 2. Leaf ultrastructural features of ten wheat genotypes (1: Masr 1, 2: Masr 2, 3: Gimmaza 9, 4: Gimmaza 11, 5: Sids 12, 6: Sids 13, 7: Sakha 93, 8: Sakha 94, 9: Shandawel 1, 10: Giza 186) each under control (a) and drought (b) conditions. Transmission electron micrographs at 4000x show chloroplasts (C) with tail (T), starch grains (S), plastoglobules (P) oleosomes (O) and cell wall (CW).

Gimmaza 11 resembled Sids 12 in containing the maximum amount of chlorophyll a, while Giza 186 was the supreme genotype when determining chlorophyll b and total chlorophyll. By calculating chlorophyll a/b ratio, Masr 2 was found to be the supreme amongst the studied genotypes. The SP analysis irrespective of genotype showed that drought caused highly significant decrease in chlorophyll a content and chlorophyll a/b ratio with highly significant increase in chlorophyll b content of the studied wheat plants. The 1WCR analysis at $p \leq 0.05$ revealed that the decrease recorded in chlorophyll a content was significant in Masr, Gimmaza 9, Sids 13 and Giza 186 only. Drought increased chlorophyll b content in all genotypes except for Masr 2 (non-significant decrease) but such increase was non-significant only in Gimmaza 11, Sids 12 and Sakha 93. Drought also increased total chlorophyll content in all genotypes except Masr 2 (non-significant decrease) and such increase was mostly significant. Regarding chlorophyll a/b ratio, drought decreased its value in all genotypes except for

Masr 2 (non-significant increase) and the decrease was significant only in Masr 1, Gimmaza 9 and Sids 13 (**Table 3**).

Table 3. Effect of drought on leaf photosynthetic pigments of ten wheat genotypes.

		hlorophyll a (µg mg^{-1})	Chlorophyll b (µg mg^{-1})	Carotenoids (µg mg^{-1})	Total Chlorophyll (µg mg^{-1})	Chlorophyll (a/b)	Carotenoids/ Total Chlorophyll	CSI (%)
Factor AB: Genotype × Watering Level								
Masr 1	Control		$0.874^{fg} \pm 0.007$	$0.447^{fgh} \pm 0.003$	$1.946^{gh} \pm 0.007$	$1.227^{cd} \pm 0.009$	$0.230^{fg} \pm 0.001$	$100^{de} \pm 0$
	Drought	$1.068^{cde} \pm 0.005$	$1.039^{bc} \pm 0.258$	$0.505^{cde} \pm 0.110$	$2.108^{bcd} \pm 0.262$	$1.078^{efg} \pm 0.302$	$0.237^{ef} \pm 0.025$	$108^{c} \pm 13$
Masr 2	Control	$1.071^{abc} \pm 0.000$	$0.837^{fg} \pm 0.002$	$0.422^{hi} \pm 0.024$	$1.908^{gh} \pm 0.002$	$1.280^{bc} \pm 0.004$	$0.221^{gh} \pm 0.012$	$100^{de} \pm 0$
	Drought	$1.067^{ef} \pm 0.000$	$0.792^{gh} \pm 0.017$	$0.402^{i} \pm 0.006$	$1.858^{hi} \pm 0.018$	$1.347^{ab} \pm 0.028$	$0.216^{h} \pm 0.001$	$97^{e} \pm 1$
Gimmaza 9	Control	$1.073^{a} \pm 0.004$	$0.901^{ef} \pm 0.002$	$0.486^{def} \pm 0.003$	$1.974^{fg} \pm 0.003$	$1.190^{cde} \pm 0.006$	$0.246^{bcde} \pm 0.001$	$100^{de} \pm 0$
	Drought	$1.068^{def} \pm 0.000$	$1.035^{bc} \pm 0.002$	$0.506^{cde} \pm 0.003$	$2.103^{bcd} \pm 0.003$	$1.032^{ghi} \pm 0.002$	$0.241^{de} \pm 0.002$	$107^{c} \pm 1$
Gimmaza 11	Control	$1.073^{a} \pm 0.002$	$0.986^{cde} \pm 0.005$	$0.523^{bcd} \pm 0.007$	$2.058^{def} \pm 0.004$	$1.088^{efg} \pm 0.008$	$0.254^{ab} \pm 0.003$	$100^{de} \pm 0$
	Drought	$1.072^{ab} \pm 0.003$	$1.007^{cd} \pm 0.002$	$0.504^{cde} \pm 0.000$	$2.078^{cde} \pm 0.004$	$1.064^{fgh} \pm 0.004$	$0.242^{cde} \pm 0.000$	$101^{de} \pm 0$
Sids 12	Control	$1.073^{a} \pm 0.002$	$0.986^{cde} \pm 0.005$	$0.523^{bcd} \pm 0.007$	$2.058^{def} \pm 0.004$	$1.088^{efg} \pm 0.008$	$0.254^{ab} \pm 0.003$	$100^{de} \pm 0$
	Drought	$1.072^{ab} \pm 0.003$	$1.007^{cd} \pm 0.002$	$0.512^{bcd} \pm 0.013$	$2.078^{cde} \pm 0.004$	$1.064^{fgh} \pm 0.004$	$0.246^{bcde} \pm 0.006$	$101^{de} \pm 0$
Sids 13	Control	$1.071^{abcd} \pm 0.002$	$0.857^{fg} \pm 0.000$	$0.439^{ghi} \pm 0.002$	$1.928^{gh} \pm 0.001$	$1.250^{bcd} \pm 0.003$	$0.228^{fg} \pm 0.002$	$100^{de} \pm 0$
	Drought	$1.066^{efg} \pm 0.001$	$1.120^{ab} \pm 0.100$	$0.526^{bcd} \pm 0.002$	$2.186^{ab} \pm 0.010$	$0.952^{ij} \pm 0.008$	$0.241^{de} \pm 0.002$	$113^{b} \pm 1$
Sakha 93	Control	$1.067^{efg} \pm 0.002$	$0.985^{cde} \pm 0.011$	$0.533^{abc} \pm 0.002$	$2.051^{def} \pm 0.009$	$1.082^{efg} \pm 0.014$	$0.260^{a} \pm 0.002$	$100^{de} \pm 0$
	Drought	$1.064^{fgh} \pm 0.001$	$1.077^{bc} \pm 0.005$	$0.539^{abc} \pm 0.003$	$2.141^{bcd} \pm 0.005$	$0.988^{ghij} \pm 0.004$	$0.252^{abc} \pm 0.000$	$104^{cd} \pm 1$
Sakha 94	Control	$1.066^{efg} \pm 0.000$	$0.834^{fg} \pm 0.010$	$0.469^{efg} \pm 0.004$	$1.901^{gh} \pm 0.010$	$1.278^{bcd} \pm 0.016$	$0.246^{bcde} \pm 0.000$	$100^{de} \pm 0$
	Drought	$1.065^{efg} \pm 0.001$	$0.988^{cde} \pm 0.004$	$0.527^{bcd} \pm 0.002$	$2.054^{def} \pm 0.003$	$1.078^{efg} \pm 0.005$	$0.256^{ab} \pm 0.000$	$108^{c} \pm 0$
Shandawel 1	Control	1.063^{ghi}	$0.732^{h} \pm$	$0.453^{fgh} \pm$	$1.795^{i} \pm$	$1.452^{a} \pm 0.003$	$0.252^{abc} \pm 0.002$	$100^{de} \pm 0$

		± 0.001	0.000	0.003	0.001			
	Drought	1.060^{i} ± 0.001	1.104^{b} ± 0.005	0.550^{ab} ± 0.002	2.164^{bc} ± 0.004	0.960^{hij} ± 0.006	0.254^{ab} ± 0.000	120^{a} ± 1
Giza 186	Control	1.068^{bcde} ± 0.002	0.915^{def} ± 0.007	0.512^{bcd} ± 0.003	1.984^{efg} ± 0.006	1.166^{def} ± 0.010	0.258^{a} ± 0.001	100^{de} ± 0
	Drought	1.061^{hi} ± 0.001	1.212^{a} ± 0.005	0.571^{a} ± 0.002	2.273^{a} ± 0.006	0.875^{j} ± 0.003	0.251^{abcd} ± 0.000	114^{b} ± 1
Factor A: Genotype								
Masr 1		1.072^{ab} ± 0.004	0.957^{cd} ± 0.187	0.479^{c} ± 0.076	2.027^{bc} ± 0.188	1.153^{bcd} ± 0.208	0.234^{c} ± 0.016	104^{bcd} ± 9
Masr 2		1.069^{b} ± 0.003	0.814^{e} ± 0.027	0.412^{d} ± 0.019	1.883^{d} ± 0.030	1.313^{a} ± 0.0409	0.219^{d} ± 0.008	99^{e} ± 2
Gimmaza 9		1.070^{ab} ± 0.004	0.968^{bcd} ± 0.073	0.496^{bc} ± 0.011	2.038^{bc} ± 0.071	1.111^{cde} ± 0.087	0.244^{b} ± 0.003	103^{cd} ± 4
Gimmaza 11		1.072^{a} ± 0.002	0.996^{abc} ± 0.012	0.514^{ab} ± 0.011	2.068^{ab} ± 0.011	1.076^{def} ± 0.014	0.248^{ab} ± 0.007	101^{de} ± 1
Sids 12		1.072^{a} ± 0.002	0.996^{abc} ± 0.012	0.517^{ab} ± 0.011	2.068^{ab} ± 0.011	1.076^{def} ± 0.014	0.250^{ab} ± 0.006	101^{de} ± 1
Sids 13		1.068^{b} ± 0.003	0.989^{bc} ± 0.144	0.483^{c} ± 0.048	2.057^{b} ± 0.142	1.101^{cdef} ± 0.163	0.234^{c} ± 0.007	107^{abc} ± 7
Sakha 93		1.065^{c} ± 0.002	1.031^{ab} ± 0.051	0.536^{a} ± 0.004	2.096^{ab} ± 0.050	1.035^{ef} ± 0.053	0.256^{a} ± 0.005	102^{de} ± 2
Sakha 94		1.066^{c} ± 0.000	0.911^{d} ± 0.085	0.498^{bc} ± 0.032	1.977^{c} ± 0.084	1.178^{bc} ± 0.110	0.251^{a} ± 0.006	104^{bcd} ± 4
Shandawel 1		1.062^{d} ± 0.002	0.918^{d} ± 0.204	0.501^{bc} ± 0.053	1.980^{c} ± 0.202	1.206^{b} ± 0.270	0.253^{a} ± 0.001	110^{a} ± 11
Giza 186		1.065^{c} ± 0.004	1.064^{a} ± 0.163	0.542^{a} ± 0.033	2.128^{a} ± 0.159	1.021^{f} ± 0.160	0.255^{a} ± 0.004	107^{ab} ± 8
Degree of Significance		***	***	***	***	***	***	***
Factor B: Watering Level								
Control		1.070^{a} ± 0.004	0.891^{b} ± 0.079	0.481^{a} ± 0.039	1.960^{b} ± 0.081	1.210^{a} ± 0.111	0.245^{a} ± 0.014	100^{b} ± 0
Drought		1.066^{b} ± 0.004	1.038^{a} ± 0.126	0.514^{b} ± 0.052	2.104^{a} ± 0.125	1.044^{b} ± 0.145	0.244^{a} ± 0.013	107^{a} ± 8
Degree of Significance		***	***	***	***	***	ns	***
Impact Index (%)		-1	16	7	7	-14	-0.4	7

Data listed represent mean values ± standard deviation. Different superscript letters refer to significant variation at $p \leq 0.05$. Low, medium and high degree of significance is indicated by *, ** and *** while non-significant difference is abbreviated as ns

Matching these results, Pandey et al. [42] and Aref et al. [43] recorded marked reduction in chlorophyll a content of different plants facing stress. The decrease in chlorophyll a content as a result of stress could be ascribed to: (i) pigment degradation by accumulated ROS [17], (ii) suppression of pigment synthesis [44], (iii) pigment breakdown under increased chlorophyllase activity [45], and/or, (iv) interference with the de novo synthesis of proteins, the structural component of chlorophyll [46]. In addition, less availability of certain elements, especially Mg and Fe, and/or insufficient plant ability to absorb and/or translocate them may decrease pigment content. Also, stress-induced increase in chlorophyll b content was reported elsewhere [47,48]. As a mechanism of plant resistance to drought, chlorophyll b content may sometimes increase to allow more efficient photosynthesis [34]. From another point of view, the increase in chlorophyll b content in response to drought can be attributed to the enhanced production of chlorophyll b, decrease in its degradation and/or conversion of chlorophyll a to chlorophyll b. Matching this assumption, it was documented that portion of chlorophyll a can be converted under certain conditions to chlorophyll b by chlorophyllide a oxygenase [49]. In those cases where chlorophyll a content decreased with increased chlorophyll b content, the increase in total chlorophyll content can be attributed to the increased chlorophyll b. In this regard, the recorded drought-induced change in total chlorophyll content of the studied genotypes was found to be very strongly correlated with that in chlorophyll b content (r=0.99) **(Figure 1)**. Lower chlorophyll a/b ratio in stressed plants was suggested by Parida et al. [50] to indicate serious stress impact on the light harvesting systems. On contrary, Cicek and Cakirlar [51] supposed that lower chlorophyll a/b ratio under stressful conditions may be a strategy to acclimate to stress. In this connection, the recorded drought-induced decrease in chlorophyll a/b ratio of the ten genotypes was found to be negatively correlated with that in chlorophyll b (r=-0.98) and total chlorophyll (r=-0.99) contents **(Figure 1)**.

According to SP analysis depending on genotype only, Giza 186 was the supreme genotype when determining carotenoids content, carotenoids/total chlorophyll ratio and chlorophyll stability index (CSI). The SP analysis irrespective of genotype showed that drought caused non-significant change in carotenoids/total chlorophyll ratio, while it caused highly significant increase in carotenoids content and CSI. Results of 1WCR ANOVA revealed that drought increased carotenoids content in all genotypes except for Masr 2, Gimmaza 11 and Sids 12 (non-significant decrease) and such increase was non-significant only in Gimmaza 9 and Sakha 93. Drought also non-significantly decreased the calculated carotenoids/total chlorophyll ratio in all genotypes except for Masr 1, Sids 13, Sakha 94 and Shandawel 1 (significant or non-significant increase). Most importantly, CSI significantly increased by drought in all genotypes except for Gimmaza 11, Sids 12 and Sakha 93 where it non-significantly increased, and also for Masr 2 where it non-significantly decreased all at $p \leq 0.05$ **(Table 3)**. The increase in carotenoids content under stress was reported in other studies [52,53]. In addition, the extent of increase in carotenoids content recorded herein under stress was higher than that in chlorophyll b content; resulting in increased ratio of carotenoids/total chlorophyll. It was assumed that the role of carotenoids under stress conditions is very effective, since carotenoids act for: (i) light harvesting via singlet state energy transfer, (ii) photo-protection via the quenching of chlorophyll triplet state, (iii) singlet oxygen scavenging, (iv) excess energy dissipation, and, (v) plastid structural stabilizing [54]. For that, the recorded drought-induced change in carotenoids/total chlorophyll ratio in the ten genotypes was found to be strongly correlated with the change in carotenoids content (r=0.77) **(Figure 1)**. Regarding CSI, which indicates pigment maintenance under stress, higher values of such index refer to more availability of chlorophyll; helping plants withstand stress by increasing photosynthetic rate and dry matter production [55]. In this context, the recorded drought-induced change in CSI of the tested genotypes was found to be positively correlated with the drought-induced change in chlorophyll b and total chlorophyll contents (r=1 for each), but negatively correlated with the calculated chlorophyll a/b ratio (r=-0.99) **(Figure 1)**.

Gas exchange measurements may greatly account for changes in plant photosynthetic efficiency as a result of genotypic and environmental factors. In this regard, photosynthesis rate (A) is expected to be among the primary criteria affected by drought. SP analysis in **Table 4** cleared that drought caused significant decrease in A; with the genotype Sids 13 showing the maximum A value. Also, 1WCR analysis confirmed that drought caused significant decrease in A of almost all the studied genotypes. Under water deficit conditions, the rate of photosynthesis is usually decreased due to alterations in photosynthetic metabolism either by stomatal or non-stomatal limitations. The stomatal limitations occur as a result of reduced CO_2 availability to the leaves with stomata being closed. Although stomatal closure in response to drought could be considered as the most important mechanism for protecting tissues against dehydration, it usually results in decreased CO_2 assimilation. Non-stomatal limitations however could be attributed to: (i) reduction of CO_2 availability caused by diffusion limitations through the mesophyll, (ii) damage of leaf ultrastructure, (iii) inhibition of enzymes activity particularly ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), and/or, (iv) CO_2 low permeability due to the adverse effect of dehydration on leaf cuticles, cell walls and plasma membranes [56,57]. A result highly similar to that in A was observed herein for the rate of transpiration rate (E) **(Table 4)**. The observed reduction in E in response to drought matches that obtained elsewhere [58,59]. In this context,

Table 4. Effect of drought on leaf gas exchange parameters of ten wheat genotypes.

		A (µmol m^{-2} s^{-1})	*E* (mmol m^{-2} s^{-1})	*p*WUE (µmol $mmol^{-1}$)	g*s* (mmol m^{-2} s^{-1})	G*m* (µmol mol^{-1})	C*i*/g*s* (µmol $mmol^{-1}$ m^2 s)	C*i* (µmol mol^{-1})	L*s*
Factor AB: Genotype × Watering Level									
Masr 1	Control	$7.1^{cd} \pm 0.5$	$1.3^{e} \pm 0.1$	$5.7^{bcdef} \pm 0.7$	$63^{cde} \pm 6$	$0.038^{cde} \pm 0.003$	$3.0^{efgh} \pm 0.4$	$187^{cd} \pm 10$	$0.48^{bc} \pm 0.03$
	Drought	$4.6^{g} \pm 0.3$	$1.1^{ef} \pm 0.1$	$4.3^{fg} \pm 0.0$	$40^{fghi} \pm 1$	$0.022^{ghij} \pm 0.000$	$5.2^{bc} \pm 0.2$	$208^{bcd} \pm 7$	$0.42^{bcd} \pm 0.02$
Masr 2	Control	$3.6^{h} \pm 0.1$	$0.8^{fg} \pm 0.0$	$4.5^{efg} \pm 0.1$	$20^{j} \pm 1$	$0.021^{hij} \pm 0.001$	$8.6^{a} \pm 0.3$	$172^{de} \pm 6$	$0.52^{ab} \pm 0.02$
	Drought	$4.5^{g} \pm 0.3$	$1.4^{cde} \pm 0.5$	$3.5^{g} \pm 1.0$	$57^{cdef} \pm 29$	$0.022^{ghij} \pm 0.002$	$4.2^{cde} \pm 1.6$	$204^{bcd} \pm 2$	$0.44^{bcd} \pm 0.01$
Gimmaza 9	Control	$11.1^{a} \pm 1.0$	$1.7^{bc} \pm 0.4$	$6.5^{bc} \pm 0.7$	$67^{cd} \pm 12$	$0.076^{a} \pm 0.009$	$2.3^{fgh} \pm 0.4$	$147^{e} \pm 7$	$0.59^{a} \pm 0.02$
	Drought	$5.8^{f} \pm 0.1$	$1.0^{ef} \pm 0.0$	$5.7^{bcdef} \pm 0.3$	$33^{ghij} \pm 6$	$0.029^{efghi} \pm 0.002$	$6.2^{b} \pm 1.1$	$203^{bcd} \pm 8$	$0.44^{bcd} \pm 0.02$
Gimmaza 11	Control	$8.6^{b} \pm 0.2$	$1.7^{bcd} \pm 0.6$	$5.6^{cdef} \pm 1.6$	$57^{cdef} \pm 12$	$0.049^{b} \pm 0.006$	$3.3^{defgh} \pm 0.9$	$179^{cde} \pm 26$	$0.50^{abc} \pm 0.07$
	Drought	$6.2^{ef} \pm 0.2$	$1.2^{e} \pm 0.1$	$5.2^{cdef} \pm 0.4$	$43^{fgh} \pm 12$	$0.030^{efgh} \pm 0.003$	$5.2^{bc} \pm 1.9$	$211^{bc} \pm 14$	$0.41^{cd} \pm 0.04$
Sids 12	Control	$9.3^{b} \pm 0.1$	$2.0^{ab} \pm 0.2$	$4.8^{defg} \pm 0.5$	$107^{b} \pm 12$	$0.047^{bc} \pm 0.003$	$1.9^{gh} \pm 0.3$	$200^{bcd} \pm 16$	$0.44^{bcd} \pm 0.05$
	Drought	$5.9^{f} \pm 0.6$	$1.3^{e} \pm 0.0$	$4.6^{efg} \pm 0.4$	$53^{def} \pm 6$	$0.029^{efghi} \pm 0.004$	$3.9^{cdef} \pm 0.5$	$208^{bcd} \pm 6$	$0.42^{bcd} \pm 0.02$
Sids 13	Control	$11.4^{a} \pm 0.6$	$2.2^{a} \pm 0.0$	$5.2^{cdef} \pm 0.3$	$143^{a} \pm 23$	$0.045^{bcd} \pm 0.002$	$1.8^{h} \pm 0.3$	$251^{a} \pm 10$	$0.30^{e} \pm 0.03$
	Drought	$6.3^{def} \pm 0.2$	$1.1^{ef} \pm 0.0$	$5.9^{bcde} \pm 0.3$	$50^{defg} \pm 0$	$0.034^{ef} \pm 0.002$	$3.7^{cdefg} \pm 0.1$	$186^{cd} \pm 5$	$0.49^{bc} \pm 0.01$
Sakha 93	Control	$7.5^{c} \pm 0.7$	$1.3^{de} \pm 0.1$	$5.7^{bcdef} \pm 0.1$	$73^{c} \pm 6$	$0.036^{de} \pm 0.003$	$2.9^{efgh} \pm 0.2$	$209^{bcd} \pm 1$	$0.42^{bcd} \pm 0.00$
	Drought	$6.3^{def} \pm 0.7$	$1.2^{e} \pm 0.1$	$5.3^{cdef} \pm 1.0$	$47^{efg} \pm 6$	$0.029^{efgh} \pm 0.010$	$5.1^{bcd} \pm 1.5$	$234^{ab} \pm 50$	$0.35^{de} \pm 0.14$
Sakha 94	Control	$11.2^{a} \pm 0.5$	$1.7^{bc} \pm 0.0$	$6.5^{bc} \pm 0.4$	$110^{b} \pm 17$	$0.055^{b} \pm 0.005$	$1.9^{gh} \pm 0.3$	$204^{bcd} \pm 10$	$0.43^{bcd} \pm 0.03$
	Drought	$4.1^{gh} \pm 0.3$	$0.5^{g} \pm 0.0$	$9.0^{a} \pm 0.7$	$27^{hij} \pm 12$	$0.019^{ij} \pm 0.002$	$9.0^{a} \pm 2.9$	$217^{abc} \pm 14$	$0.40^{cde} \pm 0.03$
Shandawel 1	Control	$6.3^{def} \pm 0.7$	$1.3^{e} \pm 0.3$	$5.2^{cdef} \pm 1.8$	$73^{c} \pm 6$	$0.025^{fghij} \pm 0.005$	$3.4^{cdefgh} \pm 0.1$	$253^{a} \pm 25$	$0.30^{e} \pm 0.07$
	Drought	$3.4^{h} \pm 0.0$	$0.5^{g} \pm 0.1$	$6.5^{bc} \pm 1.6$	$23^{ij} \pm 6$	$0.016^{j} \pm$	$9.6^{a} \pm 1.9$	$216^{abc} \pm$	$0.40^{cde} \pm$

						0.001		14	0.03
Giza 186	Control	$8.7^{b} \pm 0.8$	$1.2^{e} \pm 0.0$	$7.1^{b} \pm 0.9$	$67^{cd} \pm 6$	$0.050^{b} \pm 0.016$	$2.7^{efgh} \pm 0.4$	$184^{cde} \pm 39$	$0.49^{abc} \pm 0.11$
	Drought	$6.9^{cde} \pm 0.2$	$1.2^{ef} \pm 0.2$	$6.1^{bcd} \pm 1.3$	$57^{cdef} \pm 12$	$0.032^{efg} \pm 0.009$	$4.1^{cdef} \pm 1.4$	$228^{ab} \pm 60$	$0.36^{de} \pm 0.17$
Factor A: Genotype									
Masr 1		$5.8^{d} \pm 1.4$	$1.2^{cd} \pm 0.1$	$5.0^{def} \pm 0.9$	$52^{de} \pm 13$	$0.030^{d} \pm 0.009$	$4.1^{bcd} \pm 1.3$	$197^{cd} \pm 14$	$0.45^{bc} \pm 0.04$
Masr 2		$4.0^{f} \pm 0.5$	$1.1^{de} \pm 0.4$	$4.0^{f} \pm 0.9$	$38^{e} \pm 27$	$0.022^{e} \pm 0.002$	$6.4^{a} \pm 2.6$	$188^{de} \pm 18$	$0.48^{ab} \pm 0.05$
Gimmaza 9		$8.5^{a} \pm 3.0$	$1.4^{abc} \pm 0.5$	$6.1^{bc} \pm 0.7$	$50^{de} \pm 20$	$0.052^{a} \pm 0.027$	$4.2^{bc} \pm 2.3$	$175^{e} \pm 31$	$0.51^{a} \pm 0.09$
Gimmaza 11		$7.4^{b} \pm 1.4$	$1.4^{ab} \pm 0.5$	$5.4^{cde} \pm 1.1$	$50^{de} \pm 13$	$0.039^{b} \pm 0.011$	$4.2^{bc} \pm 1.7$	$195^{cde} \pm 26$	$0.46^{abc} \pm 0.07$
Sids 12		$7.6^{b} \pm 1.9$	$1.6^{a} \pm 0.4$	$4.7^{ef} \pm 0.4$	$80^{b} \pm 30$	$0.038^{b} \pm 0.010$	$2.9^{cd} \pm 1.2$	$204^{bcd} \pm 12$	$0.43^{bcd} \pm 0.03$
Sids 13		$8.8^{a} \pm 2.8$	$1.6^{a} \pm 0.6$	$5.6^{cde} \pm 0.4$	$97^{a} \pm 53$	$0.040^{b} \pm 0.006$	$2.8^{d} \pm 1.1$	$219^{ab} \pm 36$	$0.39^{de} \pm 0.10$
Sakha 93		$6.9^{c} \pm 0.9$	$1.3^{bcd} \pm 0.1$	$5.5^{cde} \pm 0.7$	$60^{cd} \pm 15$	$0.033^{cd} \pm 0.008$	$4.0^{cd} \pm 1.6$	$222^{ab} \pm 35$	$0.39^{de} \pm 0.10$
Sakha 94		$7.6^{b} \pm 3.9$	$1.1^{de} \pm 0.7$	$7.7^{a} \pm 1.4$	$68^{bc} \pm 48$	$0.037^{bc} \pm 0.020$	$5.4^{ab} \pm 4.3$	$210^{bc} \pm 13$	$0.42^{cd} \pm 0.03$
Shandawel 1		$4.8^{e} \pm 1.7$	$0.9^{e} \pm 0.5$	$5.9^{bcd} \pm 1.7$	$48^{de} \pm 28$	$0.020^{e} \pm 0.006$	$6.5^{a} \pm 3.6$	$234^{a} \pm 27$	$0.35^{e} \pm 0.07$
Giza 186		$7.8^{b} \pm 1.1$	$1.2^{bcd} \pm 0.2$	$6.6^{b} \pm 1.1$	$62^{cd} \pm 10$	$0.041^{b} \pm 0.015$	$3.4^{cd} \pm 1.2$	$206^{bcd} \pm 52$	$0.43^{bcd} \pm 0.14$
Degree of Significance		***	***	***	***	***	***	***	***
Factor B: Watering Level									
Control		$8.5^{a} \pm 2.4$	$1.5^{a} \pm 0.5$	$5.7^{a} \pm 1.1$	$78^{a} \pm 34$	$0.044^{a} \pm 0.016$	$3.2^{b} \pm 2.0$	$199^{a} \pm 36$	$0.45^{a} \pm 0.10$
Drought		$5.4^{b} \pm 1.2$	$1.0^{b} \pm 0.3$	$5.6^{a} \pm 1.6$	$43^{b} \pm 15$	$0.026^{b} \pm 0.007$	$5.6^{a} \pm 2.4$	$211^{a} \pm 25$	$0.41^{a} \pm 0.07$
Degree of Significance		***	***	ns	***	***	***	ns	ns
Impact Index (%)		-36	-33	-2	-45	-41	77	6	-9

Data listed represent mean values ± standard deviation. Different superscript letters refer to significant variation at $p \leq 0.05$. Low, medium and high degree of significance is indicated by *, ** and *** while non-significant difference is abbreviated as ns

reduction in E as a result of drought was discussed by Maxwell and Johnson [60] who ascribed it to the inhibition of photosynthesis accompanied by CO_2 accumulation in guard cells with eventual reduction in stomatal conductance. Supporting this assumption, correlation among the traits of the ten genotypes revealed that the recorded drought-induced change in E was strongly correlated with that in A (r=0.83) **(Figure 1)**. From another point of view, low E may be closely related to stomatal closure under water stress. In the

current study, the decrease in E of wheat plants due to water stress can be considered as an adaptive response to cope with water deficit. In addition, photosynthetic water use efficiency (pWUE) was calculated as A/E ratio; and defined as carbon gain by photosynthesis in relation to water loss by transpiration. Data obtained herein by SP analysis showed that the genotype Sakha 94 had obviously the maximum pWUE; with drought causing non-significant decrease in pWUE of wheat plants. More details by IWCR analysis manifested that drought caused non-significant decrease in pWUE of all the studied genotypes except for Sids 13 and Shandawel 1 whose pWUE increased non-significantly by drought; while pWUE increased significantly by drought only in Sakha 94 **(Table 4)**. Matching these results, some researchers recorded that drought could decrease pWUE in some wheat genotypes but increase it in others. Reduction in pWUE in response to drought may be a result of the negative consequences of drought on A and E. However, plants with higher or at least non-significantly affected pWUE under drought conditions (as the case with all wheat genotypes in the present investigation) would have high or reasonable biomass accumulation without noticeable loss of water and thus greater ability to survive than plants with lower pWUE as previously assumed by Agnihotri et al. [61].

For stomatal conductance (g_s) that refers to the rate of water movement out of leaf into air through stomata, its pattern of change in the present study in response to drought was typically similar to that recorded for A and E; but with higher impact index **(Table 4)**. Reduction in g_s under water stress was previously reported for other plants [59,62]. In this connection, Yang et al. [63] reported that changes in g_s were closely related to leaf senescence. Furthermore, g_s decreased with increasing CO_2 under low water supplies; indicating that higher CO_2 limited the stomata opening [34]. For that, it was found that the drought-induced change in g_s in the genotypes studied herein was strongly correlated with that in A (r=0.92) and E (r=0.90) **(Figure 1)**. Matching these results, strong positive correlations was recorded by Agnihotri et al. [61] between A and g_s as well as between E and g_s. Regarding mesophyll conductance (g_m) that refers to the conductance of CO_2 transfer from leaf intercellular air-spaces into chloroplasts, a pattern of change similar to that in gs was recorded for g_m; with Sids 13 ranked in the second order after Gimmaza 9 with the maximum g_m value **(Table 4)**. Other studies showed decreased g_m in different plants suffering water stress [64,65]. In this context, it was argued that significant genotypic differences in g_m of wheat flag leaves under stress might result from the differences in RuBisCO activity and the anatomical features of leaves [64]. However, g_m may notably have an effect on both photosynthesis and water use efficiency of plants under drought situations. Olsovska et al. [66] reported that restriction of g_m may also lead to limited carboxylation efficiency. Coinciding with this assumption, the drought-induced change in g_m of the studied wheat genotypes was found to be strongly correlated with that in A (r=0.86) **(Figure 1)**.

With respect to intercellular CO_2 concentration (C_i), SP analysis revealed that drought caused non-significant increase in C_i; with Shandawel 1 followed by Sids 13 showing the maximum C_i values. Via 1WCR analysis, drought increased C_i values of most genotypes; but it decreased such value in Sids 13 and Shandawel 1. The same trend was observed for ratio of Ci to ambient CO_2 concentration (C_i/C_a) (unenclosed data) and the reverse with stomatal limitation (Ls = 1 - (Ci / Ca)) **(Table 4)**. Also, correlation matrix revealed a highly negative correlation between the drought-induced change in C_i and that in L_s (r=-0.94) **(Figure 1)**. The results reported in the present study regarding the increase in C_i under the effect of drought are to somewhat similar to those obtained by Siddique et al. [67] and Inoue et al. [68]. Adversely, the decrease in C_i as a result of drought in some genotypes was reported in other studies [69,70]. In this regard, increased C_i with decreased g_s may refer to difficulty in chloroplast efficiency; since distorted chloroplast metabolism was suggested to decrease the demand for CO_2 [71]. Therefore, Inoue et al. [68] explained that lower C_i in droughted plants might be due to higher chloroplast activity to fix CO_2 than that of well-irrigated ones. In addition, lowered C_i might be related to enhance photosynthetic rate in droughted plants; and thus it could be considered as a strategy for drought tolerance. For C_i/g_s, drought caused significant increase in this ratio with Shandawel 1 having the maximum C_i/g_s value. Drought could increase C_i/g_s in all genotypes except for Masr 2; where its C_i/g_s value decreased in response to drought **(Table 4)**. Matching such finding, Agnihotri et al. [61] while examining drought tolerance of different rice genotypes observed that plants with high A titers possessed low C_i/g_s values and vice versa with a strong negative correlation between these two parameters; taking into consideration that low mesophyll efficiency can be indicated by high C_i/g_s values. Thus, drought caused significant reduction in mesophyll efficiency of all wheat genotypes except for Masr 2. Drought-induced change in C_i/g_s of the genotypes studied herein was found to be positively correlated with the drought-induced change in pWUE (r=0.82) but negatively correlated with g_m (r=-0.79) **(Figure 1)**.

As the main photosynthetic product, carbohydrates represent one of the main organic components of the plant cell dry matter. However, the amount of carbohydrates in plants is usually affected by water stress. In the present study, SP analysis showed that the genotype Sids 13 had almost the highest amount of glucose, fructose, sucrose, trehalose, total soluble sugars, polysaccharides and total carbohydrates **(Table 5)**. Irrespective of genotype, drought caused significant increase in the amount of all the assessed carbohydrates fractions and their total amount except for polysaccharides whose amount was significantly decreased under drought. Via 1WCR data analysis in **Table 5**, drought

caused significant decrease in polysaccharides content of almost all the studied genotypes except for Sids 13 and Sakha 94 (significant increase). For the eight genotypes in which polysaccharides content decreased by drought, four of them (Masr 1, Gimmaza 11, Sids 12 and Giza 186) exhibited significant decrease in polysaccharides content while in the other four, the recorded decrease was non-significant. Similarly, Alsokari [21] recorded that water stress could reduce polysaccharides amount in cowpea plants. The stress-induced reduction in chlorophyll content along with the suppression of carbon gain and photosynthetic efficiency may account for the reduced polysaccharides amount recorded herein as a result of drought. In this regard, a strong correlation was recorded between the drought-induced decrease in polysaccharides content of the ten genotypes and the drought-induced change in their carotenoids content (r=0.77) as well as the calculated carotenoids/total chlorophyll ratio (r=1) **(Figure 1)**. From another point of view, the recoded decrease in polysaccharides content may be a tolerance feature in terms of its carbohydrate subunits hold back for growth as suggested by Chaves et al. [73].

Table 5. Effect of drought on leaf carbohydrates (mg g^{-1} d wt) of ten wheat genotypes.

		Glucose	Fructose	Sucrose	Total Soluble Sugars	Trehalose	Poly-saccharides	Total Carbo-hydrates
Factor AB: Genotype × Watering Level								
Masr 1	Control	$0.94^{m} \pm 0.01$	$0.64^{j} \pm 0.01$	$2.74^{n} \pm 0.01$	$5.78^{m} \pm 0.05$	$34.7^{l} \pm 0.1$	$42.4^{b} \pm 0.9$	$82.8^{k} \pm 0.9$
	Drought	$1.33^{j} \pm 0.09$	$0.95^{f} \pm 0.01$	$3.98^{k} \pm 0.05$	$8.35^{h} \pm 0.05$	$35.6^{k} \pm 0.2$	$37.8^{f} \pm 0.1$	$81.7^{kl} \pm 0.2$
Masr 2	Control	$1.15^{l} \pm 0.05$	$0.85^{h} \pm 0.01$	$3.85^{k} \pm 0.08$	$7.18^{jk} \pm 0.03$	$37.8^{j} \pm 0.3$	$39.2^{d} \pm 0.5$	$84.2^{j} \pm 0.3$
	Drought	$1.24^{k} \pm 0.10$	$0.87^{gh} \pm 0.01$	$3.41^{l} \pm 0.01$	$6.35^{l} \pm 0.07$	$36.1^{k} \pm 0.2$	$38.5^{def} \pm 0.5$	$81.0^{l} \pm 0.4$
Gimmaza 9	Control	$1.71^{h} \pm 0.03$	$0.81^{i} \pm 0.01$	$3.47^{l} \pm 0.01$	$8.24^{h} \pm 0.05$	$43.7^{h} \pm 0.4$	$41.4^{c} \pm 0.8$	$93.3^{g} \pm 1.2$
	Drought	$1.69^{h} \pm 0.01$	$0.99^{e} \pm 0.02$	$5.59^{e} \pm 0.03$	$11.02^{d} \pm 0.08$	$48.4^{f} \pm 0.8$	$41.1^{c} \pm 0.8$	$100.5^{d} \pm 0.1$
Gimmaza 11	Control	$1.57^{i} \pm 0.04$	$0.88^{g} \pm 0.01$	$5.25^{g} \pm 0.36$	$9.18^{g} \pm 0.01$	$47.8^{f} \pm 0.4$	$41.3^{c} \pm 0.7$	$98.3^{e} \pm 0.7$
	Drought	$1.92^{ef} \pm 0.04$	$1.12^{b} \pm 0.04$	$8.69^{a} \pm 0.03$	$17.28^{a} \pm 0.20$	$52.5^{d} \pm 0.2$	$37.9^{fg} \pm 0.4$	$107.4^{b} \pm 0.6$
Sids 12	Control	$1.88^{fg} \pm 0.03$	$1.02^{d} \pm 0.01$	$3.14^{m} \pm 0.00$	$6.39^{l} \pm 0.08$	$35.5^{kl} \pm 0.6$	$36.8^{gh} \pm 1.1$	$78.6^{n} \pm 0.6$
	Drought	$2.21^{b} \pm 0.02$	$1.00^{de} \pm 0.07$	$4.54^{j} \pm 0.04$	$10.67^{e} \pm 0.23$	$49.5^{e} \pm 1.1$	$35.8^{i} \pm 1.0$	$96.0^{f} \pm 0.7$
Sids 13	Control	$2.10^{cd} \pm 0.03$	$1.02^{d} \pm 0.04$	$5.97^{d} \pm 0.02$	$11.58^{c} \pm 0.25$	$52.9^{d} \pm 0.4$	$41.0^{c} \pm 0.1$	$105.5^{c} \pm 0.6$
	Drought	$2.92^{a} \pm 0.04$	$1.16^{a} \pm 0.03$	$7.44^{b} \pm 0.02$	$15.35^{b} \pm 0.35$	$61.5^{a} \pm 0.1$	$44.7^{a} \pm 0.8$	$121.6^{a} \pm 1.2$
Sakha 93	Control	$2.21^{b} \pm 0.04$	$0.94^{f} \pm 0.01$	$4.59^{ij} \pm 0.04$	$5.38^{n} \pm 0.22$	$38.4^{j} \pm 0.5$	$36.0^{hi} \pm 0.1$	$79.8^{m} \pm 0.4$
	Drought	$1.92^{ef} \pm 0.02$	$1.14^{ab} \pm 0.01$	$3.91^{k} \pm 0.02$	$7.27^{jk} \pm 0.08$	$34.7^{l} \pm 0.5$	$35.9^{hi} \pm 0.1$	$77.9^{n} \pm 0.4$
Sakha 94	Control	$1.99^{e} \pm 0.01$	$0.89^{g} \pm 0.02$	$5.52^{ef} \pm 0.15$	$9.07^{g} \pm 0.06$	$54.9^{b} \pm 0.3$	$33.3^{j} \pm 0.4$	$97.3^{e} \pm 0.7$
	Drought	$2.14^{cd} \pm 0.05$	$1.07^{c} \pm 0.02$	$6.98^{c} \pm 0.03$	$9.78^{f} \pm 0.28$	$53.9^{c} \pm 0.4$	$36.6^{ghi} \pm 0.3$	$100.3^{d} \pm 0.4$

handawel 1	Control	$1.81^{g} \pm 0.04$	$0.81^{i} \pm 0.01$	$5.67^{e} \pm 0.02$	$6.18^{l} \pm 0.08$	$46.1^{g} \pm 0.6$	$39.1^{de} \pm 0.01$	$91.4^{h} \pm 0.7$
	Drought	$2.16^{bc} \pm 0.05$	$0.90^{g} \pm 0.02$	$5.10^{h} \pm 0.01$	$7.80^{i} \pm 0.23$	$41.3^{i} \pm 0.5$	$38.1^{ef} \pm 0.7$	$87.2^{i} \pm 0.8$
Giza 186	Control	$1.73^{h} \pm 0.00$	$0.88^{gh} \pm 0.01$	$5.37^{fg} \pm 0.04$	$7.36^{j} \pm 0.14$	$46.1^{g} \pm 0.3$	$38.4^{def} \pm 0.7$	$91.9^{h} \pm 1.1$
	Drought	$2.09^{d} \pm 0.01$	$080^{i} \pm 0.01$	$4.71^{i} \pm 0.01$	$7.00^{k} \pm 0.14$	$43.1^{h} \pm 0.3$	$36.3^{hi} \pm 0.2$	$86.4^{i} \pm 0.4$
Factor A: Genotype								
Masr 1		$1.14^{g} \pm 0.22$	$0.80^{h} \pm 0.17$	$3.36^{j} \pm 0.68$	$7.06^{f} \pm 1.41$	$35.2^{i} \pm 0.5$	$40.1^{c} \pm 2.6$	$82.3^{g} \pm 0.8$
Masr 2		$1.20^{f} \pm 0.08$	$0.86^{f} \pm 0.01$	$3.63^{i} \pm 0.25$	$6.76^{g} \pm 0.45$	$36.9^{h} \pm 1.0$	$38.9^{de} \pm 0.6$	$82.6^{g} \pm 1.8$
Gimmaza 9		$1.70^{e} \pm 0.02$	$0.90^{e} \pm 0.10$	$4.53^{f} \pm 1.16$	$9.63^{c} \pm 1.52$	$46.1^{d} \pm 2.6$	$41.2^{b} \pm 0.7$	$96.9^{d} \pm 4.0$
Gimmaza 11		$1.75^{e} \pm 0.19$	$1.00^{cd} \pm 0.14$	$6.97^{a} \pm 1.90$	$13.23^{b} \pm 4.44$	$50.1^{c} \pm 2.6$	$39.4^{cd} \pm 2.1$	$102.8^{b} \pm 5.0$
Sids 12		$2.04^{b} \pm 0.18$	$1.01^{c} \pm 0.02$	$3.84^{h} \pm 0.77$	$8.53^{e} \pm 2.35$	$42.5^{g} \pm 7.7$	$36.3^{g} \pm 1.1$	$87.3^{f} \pm 9.5$
Sids 13		$2.51^{a} \pm 0.45$	$1.09^{a} \pm 0.09$	$6.70^{b} \pm 0.81$	$13.47^{a} \pm 2.08$	$57.2^{a} \pm 4.7$	$42.9^{a} \pm 2.1$	$113.5^{a} \pm 8.9$
Sakha 93		$2.07^{b} \pm 0.16$	$1.04^{b} \pm 0.11$	$4.25^{g} \pm 0.38$	$6.32^{h} \pm 1.05$	$36.6^{h} \pm 2.1$	$35.9^{g} \pm 0.1$	$78.8^{h} \pm 1.1$
Sakha 94		$2.06^{b} \pm 0.09$	$0.98^{d} \pm 0.10$	$6.25^{c} \pm 0.81$	$9.42^{d} \pm 0.43$	$54.4^{b} \pm 0.6$	$35.0^{h} \pm 1.8$	$98.8^{c} \pm 1.7$
Shandawel 1		$1.99^{c} \pm 0.20$	$0.85^{fg} \pm 0.05$	$5.38^{d} \pm 0.31$	$6.99^{f} \pm 0.90$	$43.7^{f} \pm 2.7$	$38.6^{e} \pm 0.7$	$89.3^{e} \pm 2.4$
Giza 186		$1.91^{d} \pm 0.20$	$0.84^{g} \pm 0.05$	$5.04^{e} \pm 0.36$	$7.18^{f} \pm 0.23$	$44.6^{e} \pm 1.6$	$37.4^{f} \pm 1.3$	$89.1^{e} \pm 3.1$
Degree of Significance		***	***	***	***	***	***	
Factor B: Watering Level								
Control		$1.71^{b} \pm 0.39$	$0.87^{b} \pm 0.11$	$4.56^{b} \pm 1.13$	$7.63^{b} \pm 1.85$	$43.8^{b} \pm 6.8$	$38.9^{a} \pm 2.8$	$90.3^{b} \pm 8.5$
Drought		$1.96^{a} \pm 0.46$	$1.00^{a} \pm 0.12$	$5.43^{a} \pm 1.67$	$10.09^{a} \pm 3.53$	$45.7^{a} \pm 8.7$	$38.2^{b} \pm 2.7$	$94.0^{a} \pm 13.3$
Degree of Significance		***	***	***	***	**		***
Impact Index (%)		15	14	19	32	4		-7

Data listed represent mean values ± standard deviation. Different superscript letters refer to significant variation at $p \leq 0.05$. Low, medium and high degree of significance is indicated by *, ** and *** while non-significant difference is abbreviated as ns

Table 5 also cleared that drought caused significant increase in glucose content of almost all the studied genotypes except for Gimmaza 9 and Sakha 93 (non-significant and significant decrease, respectively). Drought also increased fructose content of almost all the genotypes except for Sids 12 and Giza 186 (non-significant and significant decrease, respectively). For sucrose content, drought increased it significantly in Masr 1, Gimmaza, Sids and Sakha 94; but decreased it significantly in Masr 2, Sakha 93, Shandawel 1

and Giza 186. For trehalose content, drought increased it significantly in Masr 1, Gimmaza and Sids; but decreased it significantly in the remaining five genotypes. Matching the results recorded herein, several physiological studies documented the accumulation of various sugars in plants under limited water conditions [57,73]. Accumulation of various carbohydrates can be considered as a strategy for drought tolerance. A strong correlation between the accumulation of sugars and osmotic stress resistance had been intensively recorded [74]. Moreover, high concentration of carbohydrates along with its role in lowering water potential contributes in avoiding oxidative breakdown by ROS and preserving the structure of proteins and membranes under drought [75]. In addition, carbohydrates can act as signaling molecules for sugar-responsive genes leading to different physiological responses like defense and turgor-driven cell expansion [76]. Regarding sucrose, it can act for maintaining membrane phospholipids in the liquid-crystalline phase and preventing structural changes in soluble proteins [73]. In addition, glucose participates in cross linking with protein by a complex glycosylation reaction between amino and carbonyl groups [77]. Interestingly, it was assumed that the role of trehalose accumulation in response to water stress was very effective; since it acts as a stabilizer compound which could take part in drought tolerance. In this context, trehalose can stabilize biological structures, proteins and membrane lipids in different plants; and can also protect photosynthetic electron transport chain during water stress [78]. In the current study, a strong correlation was recorded between the drought-induced change in trehalose content of the ten wheat genotypes and that in their total carbohydrates content (r=0.95) (**Figure 1**).

It seemed very critical to summarize the overall leaf photosynthetic efficiency in relation to its agro-histological features of the surveyed wheat genotypes depending on the obtained data. For that, stress impact coefficient (SIC) and stress impact index (SII) were calculated to indicate the effect of drought on the estimated parameters. Irrespective of genotype, values of SIC showed that drought had general positive effect on both of the photosynthetic pigments (8% impact index) and carbohydrates content (9%). On contrary, drought had general negative effect on leaf agronomic features (-15%), anatomical features (-4%) as well as gas exchange parameters (-23%); with leaf potentiality for gas exchange being affected by drought more vigorously than leaf agronomic traits and finally came the anatomical features with the least affected degree. Regarding the various genotypes addressed herein, values of SII represented in **Figure 3** indicated that drought could generally exert the lowest negative effect on leaf agronomic and anatomical features in wheat genotype Giza 186 and on gas exchange in Masr 2 [79-81]. At the same time, drought could exert the highest positive effect on leaf pigments in wheat genotype Shandawel 1; and on carbohydrates contents in Sids 12.

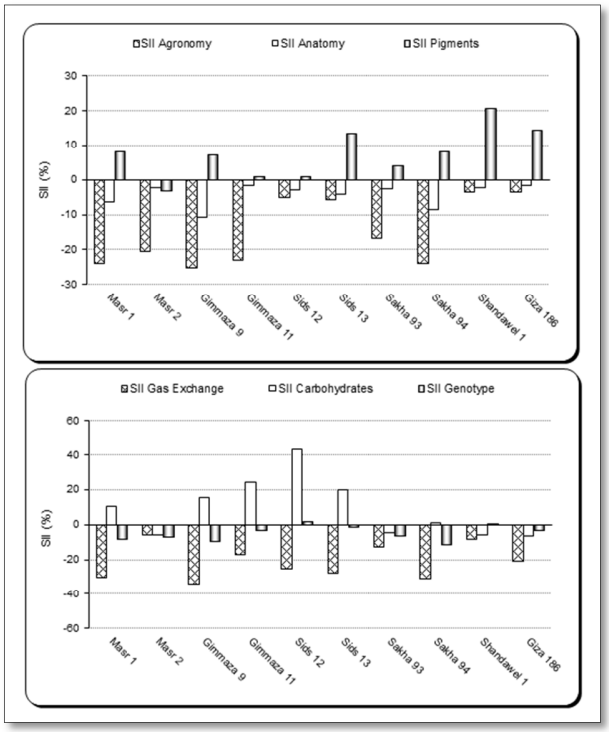

Figure 3. Stress impact index (SII; %) for different traits and genotypes of ten wheat genotypes in response to drought.

CONCLUSION

Based on the results obtained herein, wheat genotypes Sids 12, Sids 13 and Shandawel 1 may be the most droughts tolerant among the addressed genotypes on the basis of their flag leaf agro-histological features and photosynthetic machinery. Further studies are being carried out to assess the impact of drought on other physiological indices of these genotypes at booting and subsequent yield stage.

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